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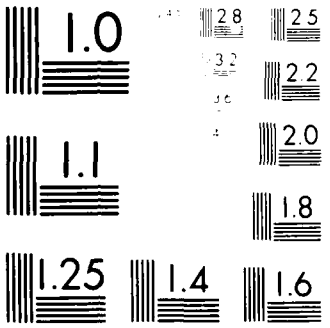
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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

This research project is investigating strategies to pharmacologically manipulate the circadian sleep-wake cycle in order to control the timing of alert function and of sleep in altered work schedule environments. In the past year we have investigated the benzodiazepines, diazepam (in hamsters) and triazolam (in squirrel monkeys), and have derived a phase response curve for each. In optically-enucleated hamsters, however, consistent phase shifts were not obtained suggesting that diazepam acts on light information-conveying pathways. Biochemical receptor binding studies are defining the benzodiazepine receptor density in various brain regions. In addition, the characterization of the circadian and homeostatic components of sleep in the squirrel monkey during sleep deprivation studies is being conducted in preparation for pharmacological manipulation with benzodiazepines.

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M.C. Moore-Ede

ANNUAL TECHNICAL REPORT

AIR FORCE OFFICE OF SCIENTIFIC RESEARCH

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During the past year we have made good progress on the various aspects of our research program investigating pharmacological methods of resetting the circadian sleep-wake cycle.

1. Rodent Chronopharmacology Screening Facility

We are currently exploring the circadian effects of benzodiazepines in hamsters at both the behavioral and biochemical level. The benzodiazepines have been shown to cause both advance and delay phase shifts in the activity rhythms of hamsters. Systemic, intraocular and intracranial injections of benzodiazepines into hamsters under various lighting conditions are probing the sites of benzodiazepine action, while the circadian phase-resetting effects are monitored by computer-recorded wheel-running activity rhythms. By administering benzodiazepines under constant light, constant dark, or optically-enucleated conditions, we hope to distinguish benzodiazepine effects on the light input pathways to the central circadian pacemaker from effects on the central pacemaker itself.

A phase response curve (PRC) to 12.5 mg/kg diazepam has been completed in sighted hamsters under constant light and this was found to be very similar to the PRC to dark pulses, suggesting that the benzodiazepines may act via light input pathways. This conclusion appears to be confirmed by the PRC we then obtained for diazepam in optically-enucleated hamsters, which is very attenuated compared to the sighted PRC. We plan to determine PRCs for benzodiazepines in sighted hamsters in constant darkness, and the effects of intraocular injections will be compared to intracranial and systemic injections to distinguish the site of action.

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In the near future we plan to supplement the behavioral monitoring of benzodiazepine effects with in vivo multiple unit activity recordings from the suprachiasmatic nuclei (SCN) (the neural generator of circadian rhythms). By recording the rhythmic gross electrical activity of the SCN under various drug treatments, we hope to dissect the pharmacology of the SCN.

Simultaneously, biochemical receptor binding studies tracing benzodiazepine receptor density rhthyms in various brain regions, as

well as autoradiographic localization of benzodiazepine receptors in the suprachiasmatic nuclei, are providing a biochemical basis for the observed behavioral effects. The retinae, cortex, hypothalamus and other brain regions are dissected from hamsters at intervals across the 24-hour day. The tissue is homogenized and incubated with tritiated benzodiazepines to characterize diurnal fluctuations in receptor density. Initial studies at a single timepoint have shown the hamster to have a receptor distribution similar to rats and other species, with highest benzodiazepine receptor density in the cortex and cerebellum, and lowest in the brain stem. In order to precisely characterize the receptor types of the SCN, hamsters are perfused at various timepoints and the brains serially sectioned through the hypothalamus and SCN. The brain sections are incubated in tritiated benzodiazepines and other compounds of interest and exposed to film for several weeks. The resulting photographic images are digitized onto a computer, where image analysis and reconstruction can pinpoint the anatomical distribution of drug receptors in the SCN.

2. Resetting of Primate Circadian Rhythms

Recent studies in the hamster indicate that the short half-life benzodiazepine, triazolam, can phase shift circadian activity rhythms in the hamster. The magnitude and direction of the phase shift depend on the circadian phase at which the drug is administered, in the same way we have shown that diazepam-induced phase shifts are phase dependent.

We are currently investigating whether triazolam has circadian phase-resetting effects in freely-ranging squirrel monkeys--a diurnal primate with a sleep-wake cycle similar to humans. All monkeys have been recorded under constant light (60 lux) in isolation chambers equipped with 1 or 2 perches connected to microswitches. When the monkeys move on or off the perches, the microswitches open and close producing pulses which are counted and stored by computer. The monkeys are recorded continuously for 3 months, during which period they receive an intraperitoneal injection of triazolam approximately every 3 weeks at a selected circadian phase.

We have tested doses ranging from .05 mg to .4 mg (in 1.0 + .1 kg monkeys). The .05 dose produces a mild sedation lasting 1.5 - 2.5 h. The .4 mg dose produces a heavy sedation for up to 8 h. We are presently concentrating our efforts on a dose of .2 mg which produces a moderate sedation for 2.5 - 3.5 h.

The results to date indicate that triazolam can phase shift monkey circadian rhythms. The effects are phase dependent and virtually the same as those observed in hamsters. The percentage of

injections producing measurable phase shifts is about 50%--less than that for hamsters. This may be because the recorded rest-activity circadian rhythm of monkeys is less precise than the wheel-running activity onset of hamsters; we are not likely to detect phase shifts of 30 - 40 min or less. The phase shifts which we have unequivocally observed range in magnitude from 1.0 to 3.0 h.

We are presently completing the phase response profile for the .2 mg dose. Five data points (injections) will be obtained at each of 8 circadian phases. We then plan to do a dose-response study in which we will test a range of doses at two circadian phases at which maximal advance and delay phase shifts are obtained.

3. Circadian and Homeostatic Regulation of Primate Sleep-Wake Cycle

In preparation for studying the mechanisms of pharmacological resetting of the primate sleep-wake cycle, we are evaluating the relative contributions of circadian factors and homeostatic recovery processes in the regulation of sleep in the squirrel monkey (Saimiri sciureus). This is being analyzed by studying sleep-wake stage organization following sleep deprivations of specific selected lengths and terminating at specific selected circadian phases. More specifically, the project will: a) determine the degree of dependence of multiple sleep characteristics including duration, timing, composition, and cortical EEG power content in different frequency ranges on the duration of prior wakefulness and on circadian phase; b) establish whether EEG power density in the delta range reflects a purely homeostatic recovery process as suggested in a recent model of human sleep organization; c) determine whether sleep-wake behavior has any feedback effects on the circadian pacemaker(s) by assessing whether sleep deprivation induces phase shifts of free-running circadian rhythms.

To sort the relative influences of circadian and homeostatic factors, sleep deprivations (SDs) of various lengths and ending at different circadian phases are performed on squirrel monkeys. The squirrel monkeys have implants which allow for continuous recording of sleep and circadian variables. Recordings began at least one full day before the SD, continued through the SD and for at least two full days afterward. SDs all begin one hour before predicted time of consolidated sleep onset (CS). Six SD lengths are used. They are divided into three pairs; within each pair the circadian phase at the end of the SD is the same but there is one extra circadian cycle of wake in one. One pair studied was 0 and 360 degrees, which ended at a time when the animal would normally be expected to go to sleep; thus increasing the effect of prior wakefulness only. The second pair was 90 and 450 degrees which released the animal from SD half-way through its predicted sleep period; the animal would be expected to fall asleep almost immediately and if circadian

influence were strong, the animal would awaken after a short bout of sleep at its usual circadian wake-up phase, whereas if reactive sleep timing were dominant, the animal would sleep longer. The third pair was 180 and 540 degrees which allowed the animal to go to sleep at the time at which it would normally awaken; circadian regulation would predict that the animal would not fall asleep, while reactive regulation would predict the animal would. Fourteen sleep deprivations in four animals have been completed. Results show that after the 0, 360, 90 and 450 degree SDs, the animals fall asleep almost immediately. After the 180 and 540 degree SDs, the animals do not sleep until their usual phase, suggesting strong circadian influence. Wake-ups are divided between ending at circadian times (one of the 90 and one of the 360 degree SDs, plus all of the 0, 180, 450 and 540 degree SDs), and the homeostatic predicted times (one of the 90 and one of the 360 degree SDs). Spectral power density from FFT analysis in the 0.5-2.0 Hz or delta range has been proposed to reflect the intensity of, or drive, for sleep; it should be higher after prolonged wake periods. In the 180 degree SD, the power density during the naps (short sleep periods after the end of the SD) is lower than that at the beginning of the next CS, despite the longer wake before the naps. In the 360 degree SD, the power density after a complete day of SD is not appreciably higher than that after normal days. These suggest that there is a circadian modulation of power density or that power density is not an accurate measure of sleep need. In the coming year we plan to investigate the effects of benzodiazepine phase-resetting drugs on sleep and EEG measures in this same preparation.

4. Personnel Associated with the Research Effort

M.C. Moore-Ede, M.D., Ph.D., Principal Investigator
R.E. Mistlberger, Ph.D., Senior Research Associate
Mr. T.A. Houpt, Graduate Student
Ms. E.B. Klerman, Graduate Student
Ms. L.K. Dewey, Research Assistant
Ms. L.C. Kilham, Administrative Assistant

5. Publications

Papers

Moore-Ede, M.C. Physiology of the circadian timing system: Predictive versus reactive homeostasis. Thirtieth Annual Bowditch Lecture. Am. J. Physiol. 19 (5): R735-R752, 1986.

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